Biochanin A: A Comprehensive Overview of its Pharmacological Properties and Therapeutic Potential

Mashael M. AlMutairi, PhD* Rania M. Magadmi* Rasheed A. Shaik** and Huda M. AlKreathy*

ABSTRACT

Mostly found in plants including red clover, chickpeas, and soy, natural occurring isoflavones include Biochanin A (BCA). Pharmacological research has been much interested in its wide spectrum of biological action and therapeutic applications. Being a phytoestrogen, BCA exhibits both oestrogenic and anti-estrogenic properties, which makes it a suitable treatment for diseases linked to hormones like menopausal symptoms, osteoporosis, and breast cancer. Its key mediator is its interaction with oestrogen receptors (ERα and ERβ), where, depending on the type of tissue, it may either mimic or impede the activity of endogenous oestrogen. Apart from its hormonal effects, BCA displays clear anti-inflammatory and antioxidant function. Modulated are important signaling pathways linked in inflammation, cell proliferation, and death: NF-κB, PI3K/AKT, and MAPK. These mechanisms help to explain both avoidance and its promise in treating chronic inflammatory diseases as well as cancer. Moreover, its antioxidant effect helps neutralize free radicals and reduce oxidative stress, a main component in the pathophysiology of many disorders including neurological ailments and cardiovascular difficulties. BCA has also demonstrated cardioprotective effects by improving lipid profiles, reducing cholesterol, and stopping the formation of atherosclerotic plaques. Its anticancer function is rather amazing as it has been shown in several cancer models to induce death, inhibit angiogenesis, and slow down tumor growth. These positive findings although insufficient data on the pharmacokinetics, absorption, and long-term safety of BCA restrict its therapeutic relevance. At last, BCA is a diverse natural substance with high medical value. Its many biological activities make it a good candidate for future research and development as a therapeutic agent addressing a range of diseases. Still, further extensive study including clinical trials is needed to totally explore its safety and efficacy in people.

INTRODUCTION

Biochanin A (BCA), a natural isoflavone, has been found to inhibit fatty acid amide hydrolase, an enzyme involved in pain regulation [1]. It has diverse pharmacological properties, including anticancer, anti-inflammatory, anti-bacterial, anti-diabetic, and anti-obesity effects [2]. BCA is present in various plants, such as soy, alfalfa, peanuts, and chickpea [3] Despite its potential, its clinical use is limited due to low bioavailability [4]. BCA has also been found to inhibit iNOS expression, p38-MAPK, and ATF-2 phosphorylation, and block NFκB nuclear translocation, demonstrating its anti-proliferative and anti-inflammatory activities [5]. In particular, it has demonstrated potential as a BACE1 inhibitor, suggesting a role in Alzheimer's disease prevention and treatment [6]. Protect against focal cerebral ischemia/reperfusion by inhibiting p38-mediated inflammatory responses [7]. Furthermore, it has been shown to have neuroprotective effects against β-amyloid-induced neurotoxicity [8]. Neuroprotective Effects of BCA against β-Amyloid-Induced Neurotoxicity in PC12 Cells via a Mitochondrial-Dependent Apoptosis Pathway [8]and to protect against lipopolysaccharide/D-galactosamine-induced acute liver injury in mice by activating the Nrf2 pathway and inhibiting NLRP3 inflammasome activation [9]. Its mechanisms of action in various disorders, including cancer, metabolic disorders, and neurological disorders, have been summarized [10]. However, its potential for drug-drug interactions and its effects on the aryl hydrocarbon receptor and cytochrome P450 1A1 in breast carcinoma cells warrant further investigation [11,12].

BCA has been shown to possess a wide range of therapeutic properties, including cardioprotective effects. It has been found to reduce oxidative stress and increase SIRT1 expression, leading to the attenuation of cardiomyopathy in type 2 diabetic rats [13]. Additionally, it has been shown to inhibit lipopolysaccharide-induced inflammation in human umbilical vein endothelial cells, suggesting its potential as a therapeutic agent for inflammatory cardiovascular disease [14]. Its antihyperlipidemic effect has also been demonstrated in streptozotocin-induced diabetic rats [15].

For many disorders, drugs of natural origin offer a major supply of rather safe and efficient therapy choices. Natural products have several pharmacological effects: antibacterial [16]; anti-inflammatory [17]; anticancer [18]; and antioxidant [19]. With their broad-spectrum biological activities, Flavonoids are a key class of natural compounds with varied chemical structures that have potent therapeutic effects [20]. Because their structure matches 17β-estradiol, soflavones are a subclass of flavonoids sometimes referred to as phytoestrogens. Mostly found within the legume family, Fabaceae, they include green beans, soybeans, and peanuts. Considered to have antiosteoporotic, anamnestic, and chemoprevention properties [21]

Chemical Structure and Properties

Chemically, BCA is identified as 5,7-dihydroxy-4'-methoxyisoflavone that is 7-hydroxyisoflavone which is substituted by an additional

* Department of Clinical Pharmacology
Faculty of Medicine, King Abdulaziz University, Saudi Arabia
E-mail: malmutairi0626@stu.kau.edu.sa
* Department of Pharmacology and Toxicology
Faculty of Pharmacy, King Abdulaziz University
Jeddah 21589, Saudi Arabia.

hydroxy group at position 5 and a methoxy group at position 4' [22]. The presence of hydroxyl groups is particularly significant for its antioxidant properties, as they can donate hydrogen atoms to neutralize free radicals [23].

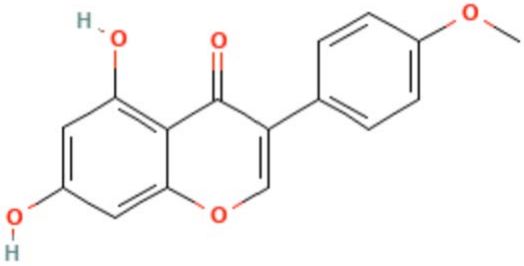

Figure1. Chemical Structure of Biochanin A

Biochanin A Plant Sources

BCA was initially isolated from the stems and leaves of *Trifolium pretense L.*, belonging to the family Leguminosae/Fabaceae, which is used utilized on largely to alleviate postmenopausal symptoms in women and to treat eczema, asthma, cough, and eye problems [24,25]. BCA can be also isolated from *Astragalus membranaceus,* the Traditional Chinese Medicine [26]. Moreover, BCA is abundant in a wide range of plants including soya bean (*Glycine max*) [15], peanuts (*Arachis hypogaea*) [27], chickpea (*Cicer arietinum*) [28,29], red clover (*Trifolium pratense*) [30], Indian rosewood (*Dalbergia sissoo*) [28], alfalfa sprouts (*Medicago sativa*) [14,31], and golden tree (*Cassia fistula*) [32].

Biochanin A Pharmacokinetics

The pharmacokinetics of oral BCA is intricate due to the interplay of two opposing effects: capacity-limited clearance and bioavailability, which affect the plasma concentration-time profiles. BCA also experiences enterohepatic recirculation. The primary metabolic processes include 4'-O-demethylation followed by conjugation, in addition to the direct conjugation of BCA. Despite BCA's limited bioavailability, enterohepatic recycling may extend the duration of exposure [33].

Biochanin A Pharmacodynamics

BCA has been demonstrated to be a viable therapeutic option in several illnesses due to its advantageous pharmacological properties and ability to modulate cell signaling. BCA has been documented to possess antioxidant, anti-inflammatory, anticancer, antibacterial, neuroprotective, hepatoprotective, and several other biological effects.

Biological Activities

Antioxidant Activity

Oxidative stress, resulting from an imbalance between free radicals and antioxidants, is implicated in numerous chronic diseases. BCA exhibits potent antioxidant activity by **scavenging reactive oxygen species (ROS)** and **upregulating endogenous antioxidant defenses** [34]. *In vitro* studies have demonstrated BCA's ability to neutralize free radicals, thereby protecting cells from oxidative damage [35].

Anti-inflammatory Effects

Chronic inflammation is a key contributor to various pathological conditions, including cardiovascular diseases and cancer. BCA has been shown to suppress the production of pro-inflammatory cytokines, such as **tumor necrosis factor-alpha (TNF-α)** and **interleukin-6 (IL-6)**, by inhibiting the **nuclear factor-kappa B (NF-κB)** signaling pathway. This modulation of inflammatory responses highlights BCA's potential as an anti-inflammatory agent.

Anticancer Properties

BCA exhibits anticancer effects through multiple mechanisms:

- **Induction of Apoptosis**: BCA promotes programmed cell death in cancer cells by activating intrinsic apoptotic pathways [36].

Neuroprotective Effects

Emerging evidence suggests that BCA offers neuroprotective benefits:

- **Oxidative Stress Reduction**: By scavenging ROS, BCA protects neuronal cells from oxidative damage[37]
- **Anti-inflammatory Actions**: BCA decreases neuroinflammation by modulating microglial activation and cytokine production [38].

Therapeutic Applications

Given its diverse biological activities, BCA holds potential in various therapeutic areas:

Cardiovascular Health: BCA's antioxidant and anti-inflammatory effects may contribute to improved cardiovascular function and reduced risk of atherosclerosis.

Cardioprotective

Inducing antioxidant and anti-inflammatory pathways in several investigations has revealed BCA to have cardioprotective benefits. By its antioxidant properties, Sharma (2019) discovered that it may stop the development of isoprenaline-induced cardiac fibrosis in rats. By lowering hyperglycemia, oxidative stress, and enhancing SIRT1 expression, [13] showed their ability to slow the development of cardiomyopathy in type 2 diabetes. By controlling lipid peroxidation, boosting antioxidants, and detoxifying enzyme systems, [39]claimed to be able to prevent isoproterenol-induced myocardial infarction in rats. By means of oxidative stress and inflammation reduction via the Nrf-2 pathway, JIR (2021)further validated these results in rats, hence mitigating obese cardiomyopathy. These findings taken together point to notable cardioprotective effectiveness of BCA. Furthermore shown to prevent arsenic-induced renal and cardiac damage is BCA [40]. As well as its function in lowering atherosclerosis by means of the suppression of lipid accumulation and inflammatory response, BCA has therapeutic efficacy in mitigating arsenic-induced renal and cardiac damage in rats [41]. By means of the TLR4/NF-κB/NLRP3 signaling pathway, BCA has also been demonstrated to reduce myocardial ischemia/reperfusion damage [42].

Metabolic Disorders: Preliminary studies suggest that BCA can enhance insulin sensitivity and modulate lipid metabolism, indicating potential benefits in managing diabetes and hyperlipidemia.

Antidiabetic

Bioflavonoids have really remarkable hypoglycemic effects [43]. It is now clear that flavonoids play the function of "insulin secretagogues or insulin-mimetic agents". Giving BCA to diabetic rats caused by streptozotocin changed their glucose metabolism and HbA1C levels dropped. Blood visfatin levels increased [44], and Salemi, 2014). By reducing free radicals brought on by hyperglycemia and fasting blood sugar, oral BCA therapy has been shown to have anti-diabetic effects in STZ diabetic rats [45]. Using db/db diabetic mice, the anti-diabetic and anti-hyperlipidemic properties of red clover extract were investigated. BCA therapy reversed elevated plasma glucose, weight gain, and HbA1C levels [15]. Perhaps outlining the anti-diabetic properties of the chemical, BCA at certain dosages enhanced insulin sensitivity,

reduced insulin resistance and glucose tolerance, and raised SIRT-1 expression [13]. Strong activation of PPAR receptors (PPARalpha/PPARgamma) at low dosages [46] shows the anti-diabetic and anti-hyperlipidemic activities of BCA. Analyzing how serum resistin and adiponectin modulate glucose metabolism in diabetes, it is evident that BCA increases the synthesis of adiponectin and thereby enhances the efficacy of insulin. BCA lowers the raised resistin levels usually seen in type-1 diabetes. BCA [47] treating diabetes-induced oxidative stress under a rat model of STZ-induced diabetic neuropathy, BCA 's effects revealed that mechanical allodynia and hyperalgesia—paw withdrawal threshold—improved following therapy. BCA may thus be the optimal course of action for diabetic neuropathy [48]. Diabetic rat retinas were examined to exclude diabetic retinopathy following BCA injection. Its anti-inflammatory and anti-angiogenic properties were demonstrated to greatly reduce retinal damage incidence [49]. Rats with type 2 diabetes mellitus were utilized to test for diabetic nephropathy, which is brought on by elevated oxidative stress and TGF-β, thereby determining the potential relevance of BCA. Reducing oxidative stress and TGF-β expression helped to greatly enhance kidney performance [50].

Antilipidemic

Hyperlipidaemia is a frequent comorbidity noticed in those with diabetes. BCA lowered small dense low-density lipoprotein cholesterol (sd-LDLC) and fasting blood sugar (FBS) when given to diabetic rats; both of these are advantageous in diabetic dyslipidemia [51]. A formulation including BCA, with or without its analogues, will be beneficial in the treatment of diabetes and diabetic cardiomyopathy as shown by the elevated levels of IGF1R (insulin-like growth factor 1 receptor), INSR (insulin receptor), and IRS2 (insulin receptor substrate 2). Rats fed HFD-activated streptozotocin once daily for sixteen weeks were administered BCA orally once daily. This indicated that whilst reducing oxidative stress and hyperglycemia, BCA might raise SRT1 expression in heart tissue. BCA might be a helpful tool for type 2 diabetics reducing the development of cardiomyopathy[13]. In diabetic rats used in a research on the consequences of diabetes and diabetic nephropathy, the administration of BCA considerably lowered the expression of transforming growth factor-β1 (TGF-β1), protease-activated receptors 2 (PAR-2) genes, and fasting blood glucose (FBG). One aspect of the anti-diabetic effect of BCA is decrease of oxidative stress. SIRT-1 influences the evolution of insulin sensitivity. Activating the PPAR gamma receptor, BCA has anti-diabetic effect. Common metabolic disorder that aggravates cardiovascular illnesses, dyslipidemia, sometimes referred to as hyperlipidemia, is typified by raised triglycerides and low-density lipoproteins [52]. Research on the link between a soy diet and hyperlipidemia is divided. Soy helps reduce raised cholesterol levels when used with traditional hyperlipidemic medications[53]. Randomized controlled studies on isoflavones in hypercholesterolemia confirm this result by showing a quite large favorable effect on triglyceride levels [54]. A tiny bit of BCA has been demonstrated to drastically reduce total and LDL cholesterol levels in mice on a high-fat diet. Level of hepatic triglyceride lipase and lipoprotein lipase is greater. Molecular docking experiments on BCA showed a significant impact in lowering cholesterol-ester transport, claims [55]. While formononetin [56] lowered LDL cholesterol levels in men, BCA did not clearly influence women. Human subject was administered plant sterol and the soy chemical BCA to evaluate LDL cholesterol levels and their effect on atherosclerosis. Co-administration of isoflavones and plant sterols has demonstrated to be very effective [10].

Overweightness

Rising as a major worldwide health issue, obesity is fast expanding into an epidemic that influences wealthy and poor nations to different degrees. Though obesity and overweight are becoming more common in modern culture, there are not any medical therapies available for either ailment right now. Thus, both researchers and healthcare systems have to give safe and effective obesity therapies first importance. In high-fat diet -induced obesity, oral BCA therapy drastically reduced the physiological alterations connected to trace element metabolism. This might occur when pathogenic processes that disturb trace elements are inhibited, maybe by adjusting hepcidin and HO-1 levels and so addressing insulin resistance and hyperglycemia. BCA raised PPAR-α expression and its regulating proteins in the liver via inducing transcriptional activation of PPAR-α in vitro. BCA therapy enhanced the recovery of metabolites associated to beta-oxidation, lipogenesis, and phosphatidylcholine synthesis in the livers of obese mice. Two enzymes connected to gluconeogenesis, pyruvate kinase, and glucose 6-phosphatase also showed inhibition by BCA. In diet-induced obesity, BCA regulated the metabolism of fat and glucose, therefore lowering metabolic abnormalities like insulin resistance and hepatic steatosis [57]. Moreover, BCA treatment demonstrated a notable therapeutic effect in obese mice as it brought the aberrant parameters virtually normal. By strengthening the Nrf-2 pathway and thereby preventing the NF-κB cascade, BCA raised the activity and mRNA expression of enzymatic antioxidants. BCA may lower inflammation and oxidative stress by activating the Nrf-2 pathway and blocking NF-κB activation, therefore reducing obesity and the cardiomyopathy associated with it [58]. BCA promotes AMPK signaling in C3H10T1/2 mesenchymal stem cells, therefore promoting the formation of brown adipocytes. Increasing lipolysis and mitochondrial biogenesis helps BCA therapy modifies the thermogenic process. BCA reduces energy consumption by increasing mitochondrial respiration in functional mitochondria preservation. The findings suggest that BCA may be a novel pharmacological treatment for obesity [59]. Leptin is a hormone that controls calories consumed and body mass. Recent research indicate that obesity development is much influenced by leptin resistance. Endoplasmic reticulum (ER) stress results from the accumulation of unfolded proteins in the ER and causes leptin resistance. Reduced glucose-regulated protein expression, altered leptin signaling brought on by ER stress, and decreased ER stress-induced neuronal cell death: BCA The findings suggest that BCA may have pharmacological properties that would lower ER stress and thereby lower leptin resistance. The cholesterol esterase inhibitory action of BCA is evaluated in this work using an in silico docking technique. [60]Sivashanmugam et al. (2013) found BCA showed cholesterol esterase inhibitory effectiveness. These molecular docking studies might guide the creation of effective cholesterol esterase inhibitors to treat obesity.

Hepatoprotective

Oxidative stress, resulting from liver exposure to various toxins like alcohols and solvents, is responsible for the development of liver injuries and diseases. Therefore, the potent antioxidant, BCA, showed potential hepatoprotective activities against various models of liver injury including carbon tetrachloride [3], and arsenic [61].

Neuroprotective

BCA exhibits neuroprotective activities against neurotoxicity induced by L-glutamate [62], lipopolysaccharide (LPS) [7], and cerebral [63], ischemia-reperfusion [63]. Moreover, it protected against the depletion of striatal dopamine in the substantia nigra of the neurochemical deficit model in rats [63,64]. BCA activates AMPK signaling in C3H10T1/2 mesenchymal stem cells, facilitating the formation of brown adipocytes. Besides it upregulated the activities of SOD, NADPH oxidase, and glutathione peroxidase (GPx) activities [65,66].

Antioxidant

BCA demonstrates an antioxidant impact through the activation of Nuclear factor erythroid 2-related factor 2 (Nrf2), which in turn induces its downstream antioxidant and cytoprotective enzymes, heme oxygenase-1 (HO-1) and NADPH quinone oxidoreductase 1[34]. BCA promotes the upregulation of the antioxidant enzymes catalase and superoxide dismutase (SOD) [61]. Furthermore, BCA exhibits significant free radical scavenging capabilities, iron chelation properties, and lipid peroxidation inhibition potential [67].

Anti-inflammatory

BCA 's anti-inflammatory impact results from its inhibition of mitogen-activated protein kinase (MAPK) and NF-κB. Furthermore suppressing the release of inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), inducible nitric oxide synthase (iNOS), nitric oxide (NO), and prostaglandin E2 (PGE2) [68]. Further research revealed that BCA reduced NF-κB activation and inhibited the production of TNF-α and IL-8 via upregulating peroxisome proliferator-activated receptor gamma (PPAR-γ), hence inducing an anti-inflammatory response [14]. Furthermore shown to be an inhibitor of inflammasome activation was BCA, which blocks the interaction between thioredoxin-interacting protein (TXNIP) and pyrin domain-containing-3 (NRRP3) and nucleotide-binding domain, leucine-rich family [9]. By reducing the activities of iNOS and cyclooxygenase-2 (COX-2) and therefore lowering the amounts of prostaglandin E2 (PGE2), BCA helps to reduce inflammation [69]. The escalating prevalence of idiopathic pulmonary fibrosis (IPF) necessitates the urgent development of novel pharmacological agents to supplant pirfenidone, which is associated with several undesirable effects [70]. BCA has been shown to markedly diminish the expression of TGF-β-regulated fibrotic genes and to decrease the expression of inflammatory markers [71]. In comparing the therapeutic efficiency of BCA with pirfenidone, BCA demonstrated superior effectiveness in alleviating pulmonary fibrosis [71]. This offers a novel approach for the management of IPF. The prevalence of acute pancreatitis (AP) is increasing, and the substantial financial burden on the healthcare system has garnered our attention [72]. Patients with acute pancreatitis have elevated blood pancreatic enzymes, which may result in multi-organ dysfunction, necessitating frequent clinic visits that significantly impair their quality of life [72]. In a murine model, BCA has been shown to diminish the migration of pathogenic Escherichia coli (E. coli) to the pancreas and to suppress TLR4-MARK/NF-κB signaling and activation of the NLRP3 inflammasome[73] therefore averting acute pancreatitis and intestinal injury. BCA may serve as a prospective medication for the treatment and prevention of AP[74].

Anticancer

Cancer is the predominant cause of mortality in those under 70 years of age in 112 of 183 nations globally. The most recent global cancer burden statistics from the World Health Organization's International Agency for Research on Cancer (IARC) indicates that there were about 19.3 million cancer diagnoses and 10 million cancer fatalities globally in 2020. Found in meals high in isoflavones, BCA is a chemical under investigation for cancer therapy. In hamster embryo cell cultures, BCA reduced carcinogen activation according to the initial report published in 1988. Research on BCA's inhibition of several kinds of tumors—including lung, prostate, gastrointestinal tract, pancreatic, breast, osteosarcoma, malignant melanoma, central nervous system tumors—showcases Strong inhibitor of cytochrome P450 (CYP), BCA is a chemopreventive drug against hydrocarbon-induced carcinogenesis. It also lowers the production of thromboxane B2 and prostaglandin E2, therefore inducing COX-2 inhibition. BCA reduces the production and activity of invading enzymes as well as offers defense against oxidative damage. By downregulating Ki-67, activating caspase-3 and caspase-9, and downregulating MMP-2 and VEGF, it can stop lung cancer cell growth. Additionally improving the effectiveness of various anticarcinogens and reducing their adverse effects is BCA. Considered as a strong chemopreventive and/or treatment agent against cancer is BCA.

BCA has been proposed as an efficacious agent for the treatment of colorectal and lung malignancies. In vitro research indicated that BCA can augment the radiotoxicity of colon tumor cells. It may also contribute to the inhibition of tumor development and immune evasion by reducing the ZEB1/PD-L1 axis [75]. BCA may block epithelial-mesenchymal transition in lung cancer and limit the proliferation rate of lung cancer cells via activating the Bcl-2 and caspase-3 pathways, as well as modulating the expression of cell cycle-related proteins [76,77]. In addition to the aforementioned diseases, BCA has demonstrated varying levels of anti-cancer efficacy in various cancer types. In head and neck malignancies, BCA can impede FaDu cell migration and proliferation by downregulating cellular signaling pathways including p38, mitogen-activated protein kinase (MAPK), NF-κB, and Akt. It may function as a prospective chemotherapeutic agent for the treatment of head and neck malignancies[78]. In breast cancer, BCA is recognized as a distinctive natural anti-cancer drug that preferentially targets cancer cells and impedes cell viability, signaling pathways, invasive enzymes, and other signaling pathways [79]. In myeloma, BCA interacts with the CD38 protein and demonstrates an antagonistic impact [80]. Furthermore, with the escalation of BCA dosage, osteosarcoma cells exhibited a decelerated growth rate, whereas normal cells shown reduced toxicity [81]. This indicates that BCA may prevent and cure osteosarcoma. In glioblastoma, BCA has demonstrated a sensitizing effect via regulating the AMPK/ULK1 pathway to suppress autophagy. BCA serves as an effective sensitizer when used in conjunction with temozolomide (TMZ) to address the limited cellular sensitivity of TMZ alone[82]. These data substantiate the potential application of BCA as an anti-cancer agent.

Antimicrobial

The advantage of using natural products with antimicrobial effects is the absence of the development of microbial resistance [83]. BCA was reported to have antiviral [84], antileishmanial [32], and antibacterial activities [85]. Moreover, it was found to have synergistic antibacterial activities with fluoroquinolones against various strains of antibiotic-resistant bacteria [86].

Hormone-related Conditions: As a phytoestrogen, BCA may alleviate menopausal symptoms and support bone health by modulating estrogen receptors.

Menopausal Symptom Relief

BCA is commonly used as a natural alternative to hormone replacement therapy (HRT) for managing menopausal symptoms , such as hot flashes, night sweats, and mood swings. Its estrogenic activity helps alleviate these symptoms without the risks associated with synthetic hormones [87–89].

Osteoporosis Prevention

By mimicking the effects of estrogen, BCA helps maintain bone density and reduce the risk of osteoporosis in postmenopausal women [90–92].

Challenges and Future Directions

Despite its therapeutic potential, several challenges hinder the clinical application of BCA. Addressing these challenges is crucial for

translating BCA's promising preclinical findings into effective clinical therapies.

Limited Clinical Data

While numerous *in vitro* and animal studies have demonstrated the therapeutic potential of BCA, **limited clinical data** is available to support its efficacy and safety in humans. Most studies have focused on preclinical models, leaving a significant gap in understanding how BCA behaves in human physiology.

- **Need for human clinical trials**: Rigorous clinical trials are essential to evaluate BCA's pharmacokinetics, optimal dosing, and therapeutic effects in humans. For example, studies on BCA's effects on menopausal symptoms or cancer prevention are still in their early stages.
- **Standardization of formulations**: Variability in the composition of BCA-containing supplements and extracts poses a challenge for clinical research. Standardized formulations are needed to ensure consistent results across studies.

Safety Profile

Although BCA is generally considered safe, its long-term safety profile remains unclear.

- **Toxicity studies**: Comprehensive toxicity studies are required to assess the safety of BCA at therapeutic doses, particularly for long-term use.
- **Potential drug interactions**: BCA may interact with other medications, such as anticoagulants or hormone therapies, necessitating further investigation into its safety in combination therapies.

Future Directions

To fully realize the therapeutic potential of BCA, future research should focus on:

- **Advanced delivery systems**: Developing innovative delivery methods, such as liposomes, micelles, or biodegradable polymers, to improve BCA's bioavailability and target specificity.
- **Mechanistic studies**: Elucidating the molecular mechanisms underlying BCA's effects, particularly its interactions with signaling pathways such as NF-κB, PI3K/Akt, and MAPK.
- **Clinical trials**: Conducting well-designed clinical trials to evaluate BCA's efficacy in treating specific conditions, such as cancer, neurodegenerative diseases, and metabolic disorders.
- **Synergistic combinations**: Exploring the potential of combining BCA with other therapeutic agents to enhance its efficacy and reduce side effects.

CONCLUSION

Natural occurring isoflavone BCA has a broad spectrum of pharmacological activities including oestrogenic, anti-inflammatory, antioxidant, anticancer, and cardioprotective action. Its promise in controlling hormone-related illnesses, chronic diseases, and cancer is highlighted by its capacity to alter important signaling pathways and interact with estrogen receptors. Though preclinical studies show great therapeutic potential, further study is needed to completely clarify its modes of action, pharmacokinetics, and long-term human safety. With more research, BCA may become a useful candidate for the creation of new therapeutic agents as it provides a natural method to solve many health issues.

Authors' Contributions: Conceptualization, HA, RM; Validation, MA, RM; Supervision, HA, RM, RS; Writing— Review and Editing; MA, RM, RS, HA

Acknowledgment: The authors would like to thank Musnad Sanad AlMutairi for his valuable support in preparing this manuscript.

Potential Conflict of Interest: None

Competing Interest: None

Acceptance Date: 15-03-2025

REFERENCES

1. Thors L, Burston JJ, Alter BJ, et al. Biochanin A, a naturally occurring inhibitor of fatty acid amide hydrolase. Br J Pharmacol 2010;160(3):549–60.
2. Yan J, Qiu P, Zhang X, et al. Biochanin A from chinese medicine: an isoflavone with diverse pharmacological properties. Am J Chin Med 2021;49(07):1623–43.
3. Breikaa RM, Algandaby MM, El-Demerdash E, Abdel-Naim AB. Biochanin A protects against acute carbon tetrachloride-induced hepatotoxicity in rats. Biosci Biotechnol Biochem 2013;77(5):909–16.
4. Raheja S, Girdhar A, Lather V, Pandita D. Biochanin A: a phytoestrogen with therapeutic potential. Trends Food Sci Technol 2018;79:55–66.
5. Kole L, Giri B, Manna SK, et al. Biochanin-A, an isoflavon, showed anti-proliferative and anti-inflammatory activities through the inhibition of iNOS expression, p38-MAPK and ATF-2 phosphorylation and blocking NFκB nuclear translocation. Eur J Pharmacol 2011;653(1–3):8–15.
6. Youn K, Park JH, Lee J, et al. The identification of Biochanin A as a potent and selective β-site app-cleaving enzyme 1 (Bace1) inhibitor. Nutrients 2016;8(10):637.
7. Wang J, He C, Wu WY, et al. Biochanin A protects dopaminergic neurons against lipopolysaccharide-induced damage and oxidative stress in a rat model of Parkinson's disease. Pharmacol Biochem Behav 2015;138:96–103.
8. Tan JW, Kim MK. Neuroprotective effects of Biochanin A against β-amyloid-induced neurotoxicity in PC12 cells via a mitochondrial-dependent apoptosis pathway. Molecules 2016;21(5):548.
9. Liu X, Wang T, Liu X, et al. Biochanin A protects lipopolysaccharide/D-galactosamine-induced acute liver injury in mice by activating the Nrf2 pathway and inhibiting NLRP3 inflammasome activation. Int Immunopharmacol 2016;38:324–31.
10. Anuranjana PV, Beegum F, Divya KP, et al. Mechanisms behind the pharmacological application of Biochanin A: a review. F1000Res 2023;12:107.
11. Han EH, Kim JY, Jeong HG. Effect of biochanin A on the aryl hydrocarbon receptor and cytochrome P450 1A1 in MCF-7 human breast carcinoma cells. Arch Pharm Res 2006;29:570–6.
12. Srinivas NR. Biochanin A: understanding the complexities in the paradoxical drug–drug interaction potential. Eur J Drug Metab Pharmacokinet 2015;40:119–125.
13. Oza MJ, Kulkarni YA. Biochanin A attenuates cardiomyopathy in type 2 diabetic rats by increasing SIRT1 expression and reducing oxidative stress. Chem Biodivers 2022;19(3):e202100591.
14. Ming X, Ding M, Zhai B, et al. Biochanin A inhibits lipopolysaccharide-induced inflammation in human umbilical vein endothelial cells. Life Sci 2015;136:36–41.
15. Harini R, Ezhumalai M, Pugalendi KV. Antihyperglycemic effect of biochanin A, a soy isoflavone, on streptozotocin-diabetic rats. Eur J Pharmacol 2012;676(1–3):89–94.

16. Strömstedt AA, Felth J, Bohlin L. Bioassays in natural product research–strategies and methods in the search for anti-inflammatory and antimicrobial activity. Phytochem Anal 2014;25(1):13–28.
17. Koeberle A, Werz O. Multi-target approach for natural products in inflammation. Drug Discov Today 2014;19(12):1871–82.
18. Marucci C, Fumagalli G, Calogero F, et al. Natural products and cancer stem cells. Curr Pharm Des 2015;21(38):5547–57.
19. López-Alarcón C, Denicola A. Evaluating the antioxidant capacity of natural products: a review on chemical and cellular-based assays. Anal Chim Acta 2013;763:1–10.
20. Mujeeb F, Bajpai P, Pathak N. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. Biomed Res Int 2014;2014(1):497606.
21. Ko KP. Isoflavones: chemistry, analysis, functions and effects on health and cancer. Asian Pac J Cancer Prev 2014;15(17):7001–10.
22. Yang Y, Yang W, Hu T, et al. Protective effect of Biochanin A on gamma radiation-induced oxidative stress, antioxidant status, apoptotic, and DNA repairing molecules in swiss albino mice. Cell Biochem Funct 2024;42(8):e70005.
23. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Sci world J 2013;2013(1):162750.
24. Kagan IA, Flythe MD. Factors affecting the separation and bioactivity of red clover (trifoliumpratense) extracts assayed against clostridium sticklandii, a ruminal hyper ammonia-producing bacterium. Nat Prod Commun 2012;7(12):1934578X1200701217.
25. Vlaisavljevic S, Kaurinovic B, Popovic M, et al. Trifolium pratense L. as a potential natural antioxidant. Molecules 2014;19(1):713–25.
26. Chen J, Ge B, Wang Y, et al. Biochanin A promotes proliferation that involves a feedback loop of microRNA-375 and estrogen receptor alpha in breast cancer cells. Cell Physiol Biochem 2015;35(2):639–46.
27. Chukwumah YC, Walker LT, Verghese M, Ogutu S. Effect of frequency and duration of ultrasonication on the extraction efficiency of selected isoflavones and trans-resveratrol from peanuts (Arachis hypogaea). Ultrason Sonochem 2009;16(2):293–9.
28. Khedgikar V, Gautam J, Kushwaha P, et al. A standardized phytopreparation from an indian medicinal plant (Dalbergia sissoo) has antiresorptive and bone-forming effects on a postmenopausal osteoporosis model of rat. Menopause 2012;19(12):1336–46.
29. Zhang L, Li Q, Yang X, Xia Z. Effects of sodium selenite and germination on the sprouting of chickpeas (Cicer arietinum L.) and its content of selenium, formononetin and biochanin A in the sprouts. Biol Trace Elem Res 2012;146:376–80.
30. Maqbool M, Shenmar K, Akther A, et al. Biochanin A chemistry, structural modifications, and therapeutic applications: an update. in: bioprospecting of tropical medicinal plants. Springer 2023. p. 789–805.
31. Lindner HR. Occurrence of anabolic agents in plants and their importance. Environ Qual Saf Suppl 1976;(5):151–8.
32. Sartorelli P, Carvalho CS, Reimão JQ, et al. Antiparasitic activity of biochanin A, an isolated isoflavone from fruits of cassia fistula (Leguminosae). Parasitol Res 2009;104:311–4.
33. Moon YJ, Sagawa K, Frederick K, et al. Pharmacokinetics and bioavailability of the isoflavone biochanin A in rats. AAPS J 2006;8:E433–42.
34. Liang F, Cao W, Huang Y, et al. Isoflavone biochanin A, a novel nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant response element activator, protects against oxidative damage in HepG2 cells. Biofactors 2019;45(4):563–74.
35. Yang Y, Yang W, Hu T, et al. Protective effect of Biochanin A on gamma radiation-induced oxidative stress, antioxidant status, apoptotic, and DNA repairing molecules in swiss albino mice. Cell Biochem Funct 2024;42(8):e70005.
36. Sarkar FH, Li Y. The role of isoflavones in cancer chemoprevention. Front Biosci 2004;9(1):2714–24.
37. Tripathi S, Mishra R, Shrivastava R, Singh G. Unveiling the neuroprotective benefits of biochanin A. in: natural molecules in neuroprotection and neurotoxicity. Elsevier 2024. p. 1307–20.
38. Berköz M, Krośniak M, Özkan-Yılmaz F, Özlüer-Hunt A. Prophylactic effect of Biochanin A in lipopolysaccharide-stimulated BV2 microglial cells. Immunopharmacol Immunotoxicol 2020;42(4):330–9.
39. Govindasami S, Uddandrao VVS, Raveendran N, Sasikumar V. Therapeutic potential of biochanin-A against isoproterenol-induced myocardial infarction in rats. Cardiovasc Hematol Agents Med Chem 2020;18(1):31–6.
40. Jalaludeen AM, Lee WY, Kim JH, et al. Therapeutic efficacy of biochanin A against arsenic-induced renal and cardiac damage in rats. Environ Toxicol Pharmacol 2015;39(3):1221–31.
41. Yu XH, Chen JJ, Deng WY, et al. Biochanin A mitigates atherosclerosis by inhibiting lipid accumulation and inflammatory response. Oxid Med Cell Longev 2020;2020:1–15.
42. Bai Y, Li Z, Liu W, et al. Biochanin A attenuates myocardial ischemia/reperfusion injury through the TLR4/NF-κB/NLRP3 signaling pathway. Acta Cir Bras 2019;34:e201901104.
43. Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutr Metab (Lond) 2015;12:1–20.
44. Azizi R, Goodarzi MT, Salemi Z. Effect of biochanin a on serum visfatin level of streptozocin-induced diabetic rats. Iran Red Crescent Med J 2014;16(9) e15454.
45. Sadri H, Goodarzi MT, Salemi Z, Seifi M. Antioxidant effects of biochanin A in streptozotocin induced diabetic rats. Braz Arch Biol Technol 2017;60:e17160741.
46. Shen P, Liu MH, Ng TY, et al. Differential effects of isoflavones, from Astragalus membranaceus and Pueraria thomsonii, on the activation of PPARα, PPARγ, and adipocyte differentiation in vitro. J Nutr 2006;136(4):899–905.
47. Morovati A. Effects of biochanin A on resistin, adiponectin and some stress oxidative markers in normal and STZ-induced diabetic rats. Arch Med Lab Sci 2018;4(2):9–16.
48. Chundi V, Challa SR, Garikapati DR, et al. Biochanin-A attenuates neuropathic pain in diabetic rats. J Ayurveda Integr Med 2016;7(4):231–7.
49. Mehrabadi ME, Salemi Z, Babaie S, Panahi M. Effect of biochanin A on retina levels of vascular endothelial growth factor, tumor necrosis factor-alpha and interleukin-1beta in rats with streptozotocin-induced diabetes. Can J Diabetes 2018;42(6):639–44.
50. Ahad A. Biochanin-A ameliorates experimental diabetic nephropathy by reducing the hyperglycemia induced oxidative stress and renal TGF-β expression. J Diabetes Metab 2013; 4:6156.
51. Ghadimi D, Goodarzi MT, Bahmani M, Khajeahmadi Z. The Effect of Biochanin A as PPAR γ agonist on LDL particles diameter and type 2 diabetic dyslipidemia. Int J Med Lab 2019;6(2):107–14.
52. Thompson GR. Management of dyslipidaemia. Heart 2004;90(8):949–55.
53. Costa RL, Summa MA. Soy protein in the management of hyperlipidemia. Ann Pharmacother 2000;34(7–8):931–5.
54. Qin Y, Niu K, Zeng Y, et al. Isoflavones for hypercholesterolaemia in adults. Cochrane Database Syst Rev 2013;(6).
55. Xue Z, Zhang Q, Yu W, et al. Potential lipid-lowering mechanisms of biochanin A. J Agric Food Chem 2017;65(19):3842–50.
56. Nestel P, Cehun M, Chronopoulos A, et al. A biochanin-enriched isoflavone from red clover lowers LDL cholesterol in men. Eur J Clin Nutr 2004;58(3):403–8.

57. Park HS, Hur HJ, Kim SH, et al. Biochanin A improves hepatic steatosis and insulin resistance by regulating the hepatic lipid and glucose metabolic pathways in diet-induced obese mice. Mol Nutr Food Res 2016;60(9)1944–55.
58. Jir A, Uddandrao VVS, Vadivukkarasi S. Biochanin A attenuates obesity cardiomyopathy in rats by inhibiting oxidative stress and inflammation through the Nrf-2 pathway. Arch Physiol Biochem 2021;129(3)788–98.
59. Rahman MS, Imran KM, Hossain M, et al. Biochanin A induces a brown-fat phenotype via improvement of mitochondrial biogenesis and activation of AMPK signaling in murine C3H10T1/2 mesenchymal stem cells. Phytother Res 2021;35(2):920–31.
60. Sivashanmugam T, Muthukrishnan S, Umamaheswari M, et al. Discovery of potential cholesterol esterase inhibitors using in silico docking studies. Bangladesh J Pharmacol 2013;8(3):223–9.
61. Jalaludeen AM, Ha WT, Lee R, et al. Biochanin A ameliorates arsenic-induced hepato-and hematotoxicity in rats. Molecules 2016;21(1):69.
62. Tan JW, Tham CL, Israf DA, et al. Neuroprotective effects of biochanin A against glutamate-induced cytotoxicity in PC12 cells via apoptosis inhibition. Neurochem Res 2013;38:512–8.
63. Guo MM, Qu SB, Lu HL, et al. Biochanin A alleviates cerebral ischemia/reperfusion injury by suppressing endoplasmic reticulum stress-induced apoptosis and p38MAPK signaling pathway in vivo and in vitro. Front Endocrinol 2021;12:646720.
64. Yu L, Wang X, Chen H, et al. Neurochemical and behavior deficits in rats with iron and rotenone co-treatment: role of redox imbalance and neuroprotection by biochanin A. Front Neurosci 2017;11:657.
65. Chen HQ, Jin ZY, Li GH. Biochanin A protects dopaminergic neurons against lipopolysaccharide-induced damage through inhibition of microglia activation and proinflammatory factors generation. Neurosci Lett 2007;417(2):112–7.
66. Wang J, Wu WY, Huang H, et al. Biochanin A protects against lipopolysaccharide-induced damage of dopaminergic neurons both in vivo and in vitro via inhibition of microglial activation. Neurotox Res 2016;30:486–98.
67. Kumari S, Elancheran R, Kotoky J, Devi R. Rapid screening and identification of phenolic antioxidants in Hydrocotyle sibthorpioides Lam. by UPLC–ESI-MS/MS. Food Chem 2016;203:521–9.
68. Zhang Y, Chen W an. Biochanin A inhibits lipopolysaccharide-induced inflammatory cytokines and mediators production in BV2 microglia. Neurochem Res 2015;40:165–71.
69. Oh JS, Cho IA, Kang KR, et al. Biochanin-A antagonizes the interleukin-1β-induced catabolic inflammation through the modulation of NFκB cellular signaling in primary rat chondrocytes. Biochem Biophys Res Commun 2016;477(4):723–30.
70. Lancaster LH, de Andrade JA, Zibrak JD, et al. Pirfenidone safety and adverse event management in idiopathic pulmonary fibrosis. Eur Respir Rev 2017;26(146).
71. Andugulapati SB, Gourishetti K, Tirunavalli SK, et al. Biochanin-A ameliorates pulmonary fibrosis by suppressing the TGF-β mediated EMT, myofibroblasts differentiation and collagen deposition in in vitro and in vivo systems. Phytomedicine 2020;78:153298.
72. Lee PJ, Papachristou GI. New insights into acute pancreatitis. Nat Rev Gastroenterol Hepatol 2019;16(8):479–96.
73. Pan X, Ye L, Ren Z, et al. Biochanin A ameliorates caerulein-induced acute pancreatitis and associated intestinal injury in mice by inhibiting TLR4 signaling. J Nutr Biochem 2023;113:109229.
74. Feng ZJ, Lai WF. Chemical and biological properties of biochanin A and its pharmaceutical applications. Pharmaceutics 2023;15(4):1105.
75. Xu J, Yang X, Pan J, et al. Biochanin A suppresses tumor progression and PD-L1 expression via inhibiting ZEB1 expression in colorectal cancer. J Oncol 2022;2022(1):3224373.
76. Wang Y, Li JJ, Chen YM. Biochanin A extirpates the epithelial-mesenchymal transition in a human lung cancer. Exp Ther Med 2018;15(3):2830–6.
77. Li Y, Yu H, Han F, et al. Biochanin A induces S phase arrest and apoptosis in lung cancer cells. Biomed Res Int 2018;2018(1):3545376.
78. Cho IA, You SJ, Kang KR, et al et al. Biochanin-A induces apoptosis and suppresses migration in FaDu human pharynx squamous carcinoma cells. Oncol Rep 2017;38(5):2985–92.
79. Sehdev V, Lai JCK, Bhushan A. Biochanin A modulates cell viability, invasion, and growth promoting signaling pathways in HER-2-positive breast cancer cells. J Oncol 2009;2009(1):121458.
80. Jaina VK, Eedara A, SVS SP, et al. Anti-cancer activity of Biochanin A against multiple myeloma by targeting the CD38 and cancer stem-like cells. Process Biochem 2022;123:11–26.
81. Hsu YN, Shyu HW, Hu TW, et al. Anti-proliferative activity of biochanin A in human osteosarcoma cells via mitochondrial-involved apoptosis. Food Chem Toxicol 2018;112:194–204.
82. Dong Q, Wang D, Li L, et al. Biochanin A sensitizes glioblastoma to temozolomide by inhibiting autophagy. Mol Neurobiol 2022;59(2):1262–72.
83. Geyid A, Abebe D, Debella A, et al. Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. J Ethnopharmacol 2005;97(3):421–7.
84. Sithisarn P, Michaelis M, Schubert-Zsilavecz M, Cinatl Jr J. Differential antiviral and anti-inflammatory mechanisms of the flavonoids biochanin A and baicalein in H5N1 influenza A virus-infected cells. Antiviral Res 2013;97(1):41–8.
85. Hanski L, Genina N, Uvell H, et al. Inhibitory activity of the isoflavone biochanin A on intracellular bacteria of genus Chlamydia and initial development of a buccal formulation. PLoS One 2014;9(12):e115115.
86. Jin H, Qi C, Zou Y, et al. Biochanin A partially restores the activity of ofloxacin and ciprofloxacin against topoisomerase IV mutation-associated fluoroquinolone-resistant Ureaplasma species. J Med Microbiol 2017;66(11):1545–53.
87. Messina M, Hughes C. Efficacy of soyfoods and soybean isoflavone supplements for alleviating menopausal symptoms is positively related to initial hot flush frequency. J Med Food 2003;6(1):1–11.
88. Krebs EE, Ensrud KE, MacDonald R, Wilt TJ. Phytoestrogens for treatment of menopausal symptoms: a systematic review. Obstet Gynecol 2004;104(4):824–36.
89. Howes LG, Howes JB, Knight DC. Isoflavone therapy for menopausal flushes: a systematic review and meta-analysis. Maturitas 2006;55(3):203–11.
90. Atkinson C, Compston JE, Day NE, et al. The effects of phytoestrogen isoflavones on bone density in women: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 2004;79(2):326–33.
91. Alekel DL, St Germain A, Peterson CT, et al. Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. Am J Clin Nutr 2000;72(3):844–52.
92. Ma DF, Qin LQ, Wang PY, Katoh R. Soy isoflavone intake increases bone mineral density in the spine of menopausal women: meta-analysis of randomized controlled trials. Clin Nutr 2008;27(1):57–64.